Effects of locoweed on serum swainsonine and selected serum constituents in sheep during acute and subacute oral/intraruminal exposure[1]

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ABSTRACT: A study was conducted to evaluate the effects of acute and subacute locoweed exposure on serum swainsonine concentrations and selected serum constituents in sheep. Thirteen mixed-breed wethers (BW = 47.5 ± 9.3 kg) were assigned randomly to 0.2, 0.4, or 0.8 mg of swainsonine·kg BW^{-1}·d^{-1} treatments. During acute (24 h) and subacute (19 d) exposure, serum swainsonine was detected in all treatments and was greatest ($P < 0.03$) in the 0.8 mg treatment. Serum alkaline phosphate (ALK-P) activity was increased ($P < 0.01$) for the 0.8 mg treatment compared with baseline (0 h) by 7 h and continued to increase throughout the initial 22 h following acute exposure to locoweed. A linear increase ($P < 0.01$) in serum ALK-P activity was noted, with the rate being 3.00 ± 0.56 U·L^{-1}·h^{-1}. Serum ALK-P activity was increased ($P < 0.05$) across treatments on d 7 over d −19, −12, 0, 1, 21, and 26; on d 14 over d −19, −12, 0, and 26; and on d 19 over d −19, −12, 0, 1, 21, and 26. By d 20, approximately 48 h after last exposure to swainsonine, serum ALK-P activities were no longer different ($P = 0.13$) than baseline (d −19, −12, and 0), and by d 26 values had generally returned to baseline. No linear ($P = 0.98$), quadratic ($P = 0.63$), or cubic effects of swainsonine with time from exposure were noted for serum aspartate aminotransferase. Similar to serum ALK-P activities, serum aspartate aminotransferase activities were increased ($P < 0.05$) across treatment levels on d 7, 14, 19, 20, 21, and 26 over those on d −19, −12, 0, and 1. Total serum Fe was decreased ($P < 0.05$) within the initial 22 h following the swainsonine exposure. On d 21 (48 h after swainsonine feeding ended), serum Fe increased to 472 mg/L. Concentrations of ceruloplasmin were lower ($P < 0.10$) on d 14 and 19 following exposure to locoweed. Recovery of ceruloplasmin levels coincided with similar changes in serum Fe. There was a linear (slope = 0.33 mg·dL^{-1}·d^{-1}; $P < 0.01$) effect with time of exposure to locoweed (i.e., swainsonine) on serum triglyceride concentrations. Rapid changes in serum ALK-P and Fe concentrations without parallel changes in other damage markers indicate that acute exposure to swainsonine induces metabolic changes that may impair animal production and health before events of cytotoxicity thought to induce clinical manifestation of locoism.

Key Words: Acute Exposure, Locoweed, Sheep, Subacute Exposure, Swainsonine

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Introduction

Several species of locoweed within the genera *Oxytropis* and *Astragalus* are toxic to livestock in the western United States, resulting in production and, ultimately, monetary losses to the producers (Nielsen et al., 1988). Consumption of locoweed results in a toxicity syndrome known as locoism, which is clinically expressed as ataxia, depression, hyperexcitability, rough hair coat, emaciation, and abortion (Broquist, 1986; Plumlee and Galey, 1994). Cytoplasmic vacuolation occurs in the pancreas, spermatozoa, epididymal epithelium, seminiferous tubule and vas deferens, liver, thyroid, nervous system, and immune cells (Sharma et al., 1984; James et al., 1986; Panter et al., 1989). The indolizidine alkaloid, swainsonine, is considered the primary toxicant in locoweeds causing cytoplasmic vacuolation and death of tissues (Molyneux and James, 1982; Tulsiani et al., 1984, 1988). The majority of stud-

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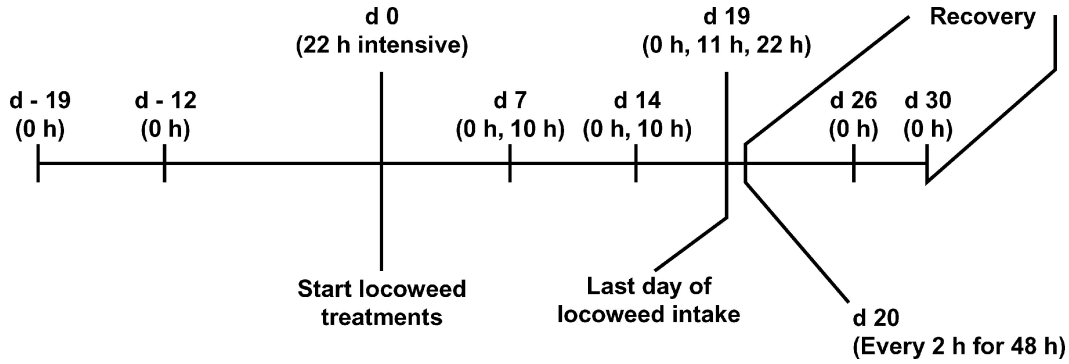

Figure 1. Time line showing the experimental sampling protocol for blood collections and the start and finish of locoweed treatment.

ies concerning locoweed intoxication have addressed swainsonine exposure rates and frequencies (late subacute and early subchronic) resulting in clinical expression of toxicity (i.e., locoism). Little attention has been paid to dosages ≤0.2 mg of swainsonine/kg of BW) at or near those reported to cause little or no toxicity (Stegelmeier et al., 1999; Taylor et al., 2000). In addition, effects of acute exposure to swainsonine have not been documented. Given the variability in consumption of locoweed by animals (Ralphs et al., 1987, 1990, 1991), one would expect both a range of dosages and frequencies of exposure in animals grazing native range. Therefore, the objective of this study was to document the effects of acute (one exposure in 24 h) and subacute (<30 d of repeated exposure) locoweed exposure under varying doses on serum swainsonine concentrations and selected serum constituents in sheep.

Materials and Methods

Animals and Housing

Thirteen mixed-breed wethers (BW = 47.5 ± 9.3 kg), fitted with ruminal and duodenal cannulas, were housed individually in metabolism crates (130 cm × 46 cm). Following a 30-d surgery recovery period, wethers were stratified by BW, randomly assigned to one of three swainsonine treatments, and subjected to a 21-d dietary adaptation period followed by a 19-d treatment period (Figure 1). The ruminal and duodenal cannulas were used as part of a companion study designed to investigate the effects of swainsonine on ruminal fermentation and duodenal and total-tract digestibility and flow of nutrients. Ruminal cannulas were used in this study to deliver locoweed as necessary. Treatments were 1) 0.2 mg of swainsonine/g BW (**0.2 mg**; n = 5), 2) 0.4 mg of swainsonine/kg BW (**0.4 mg**; n = 4), or 3) 0.8 mg of swainsonine/kg BW (**0.8 mg**; n = 4); swainsonine was delivered by feeding locoweed (*Oxytropis sericea*; 614 μg of swainsonine/g of DM). The 0.2 mg treatment is a level previously reported to have little toxicity, whereas the 0.8 mg treatment has induced both subclinical and clinical intoxication by d 14 of exposure (Taylor et al., 2000). As such, the doses selected for this experiment cover the range previously determined to be effective in sheep. Initially, 15 animals were selected for use; however, postsurgical losses necessitated an unbalanced number of animals per treatment. Five animals were placed in the 0.2 mg treatment, as this was the treatment for which we had no previous experience. Animal use was approved and followed the guidelines of the Institutional Animal Care and Use Committee.

Diets

Animals received a basal diet of blue grama and alfalfa hay during the 21-d adaptation period. During the 19-d treatment period, locoweed replaced alfalfa to deliver the treatments (Table 1). Hays were processed to 2 to 4 cm in length in a tub grinder. Locoweed (bud and flower stages) was collected as whole plants (some root portion) near Folsom, NM, allowed to air dry, and passed through a rotating blade forage chopper (1 to 1.5 cm particle length). Dry matter, CP, and ADF analyses (State Chemist Lab, NM Dept. of Agric., Las Cruces) were conducted on composite samples of the hays and locoweed. Dietary TDN values were calculated from ADF analyses (TDN = 88.9 – [0.79 × ADF%]; Moore and Undersander, 2002). Swainsonine content of locoweed was determined by an α-mannosidase inhibition assay (Taylor et al., 2000) described previously.

To avoid refusals, DMI was restricted to 1.84% of BW. Because of low palatability, locoweed was sprayed with 30 mL of a solution (approximately 115 mL of molasses and 130 g of plain salt suspended in 700 mL of water) and offered first and separately from the remainder of the diet. Sheep were allowed 15 min to consume the locoweed, and if not eaten within the 15-min period, the locoweed was moistened with water and placed in the rumen via the cannula; subsequently, the remainder of the diet was offered (0715). All diet refusals remaining after 24 h were collected and weighed. Diets were formulated to be isocaloric and isonitrogenous (Table 1), and provided 28 g of a trace mineral salt (11.5% Ca, 7.5% P, 40% NaCl, 0.5% Mg, 0.2% K, 25 ppm Cu, 3 ppm Se, and 1,825 ppm Zn) on

Table 1. Feed, swainsonine, and nutrient intakes of wethers for the 21-d adaptation and 19-d treatment periods

Item	Adaptation	Treatments[a]			Requirement[b]
		0.2 mg	0.4 mg	0.8 mg	
Alfalfa, % of DMI	2.8	2.1	1.4	0.0	—
Blue grama, % of DMI	97.2	96.1	95.0	92.9	—
Locoweed, % of DMI	0.0	1.8	3.6	7.1	—
Swainsonine, mg/kg of BW	0.0	0.2	0.4	0.8	—
DMI, % of BW	1.84	1.84	1.84	1.84	2.2
CP, g/kg of BW	2.0	2.0	2.0	2.0	2.0
TDN, g/kg of BW[c]	10.8	10.8	10.8	10.8	11.1

[a]Treatments were 0.2, 0.4, and 0.8 mg of swainsonine/kg of BW delivered by locoweed (*Oxytropis sericea*; 614 μg of swainsonine/g of DM).
[b]Maintenance requirements for a 50-kg ewe gaining 10 g/d plus 10%; NRC (1985).
[c]TDN of diets was calculated from ADF using the following: TDN = 88.9 – (0.79 × ADF%); Moore and Undersander (2002).

a daily basis. Sheep were given free access to water via automatic watering systems.

Serum Collection

Immediately following commencement of locoweed treatment on d 0 (**DAY 0**) and 48 h following treatment termination on d 20 (**DAY 20**), an intensive blood sampling event was conducted to assess 22-h serum constituent response to acute locoweed exposure and 48-h serum swainsonine disappearance from subacute locoweed exposure. Blood was collected via an indwelling jugular cannula every 30 min for the first 6 h, every hour for the next 6 h, and every 2 h for the remaining 12 h on DAY 0, and every 2 h for 48 h starting on DAY 20.

Over the course of the study, blood samples were obtained via jugular venipuncture from each animal (d –19, –14, –12, 0, 1, 7, 10, 19, 20, 21, 26, and 30) before morning feedings to assess pretreatment (d –19 to 0), treatment (d 1 to 20), and recovery (d 21 to 30) serum constituent values.

Blood samples were allowed to clot at room temperature for 1 h and centrifuged at 1,850 × *g* for 15 min. Serum was collected, transferred to a 1.5-mL polypropylene tube, and stored at <–20°C until subsequent analyses for serum constituents and swainsonine. Serum samples were analyzed for swainsonine using an α-mannosidase inhibition assay (detection limit = 6 ng/mL, intraassay CV = 13.4%; Taylor and Strickland, 2002). Additional serum constituents measured include alkaline phosphatase (**ALK-P**, Sigma Diagnostic Kit 104-LL; Sigma-Aldrich Corp., St. Louis, MO), total iron (Sigma Diagnostic Kit 565-A), unsaturated iron binding capacity (**UIBC**; Sigma Diagnostic Kit 565-A), aspartate aminotransferase (**AST**, Sigma Diagnostic Kit 505), total cholesterol (Sigma Diagnostic Kit 352), and triglycerides (Sigma Diagnostic Kit 339). Serum ceruloplasmin was measured using a modified enzymatic assay (Vogt, 1999). All Sigma diagnostic kits were modified using similar reagent ratios described for each kit, for use with a 96-well microtiter plate reader (MRX HD, Dynex, Chantilly, VA). Serum T_3 and T_4 concentrations were determined by RIA using components of a commercial kit (Diagnostic Products Corp., Los Angeles, CA) and validated for ruminant serum as described by Richards et al. (1999).

Statistical Analyses

All data were analyzed using the Proc Mixed procedure of SAS (Version 8.1, SAS Inst., Inc., Cary, NC). Model effects included treatment, time, and treatment × time, and the animal served as the experimental unit. The DAY 0 and DAY 20 intensive bleeds and serum collected over the course of the study were analyzed separately. Analyses were modified to include repeated measures using either the autoregressive (DAY 20) or spatial power law (DAY 0, daily samples) covariance structure. Linear, quadratic, and cubic effects for duration of exposure to swainsonine were tested for all serum constituents and swainsonine. Each animal served as its own control using the baseline (d –19 to 0) constituent and swainsonine values. Preplanned pairwise contrasts were used to separate means. Distribution and/or elimination rates for serum constituents and swainsonine were estimated using a curve-stripping program (PK Solutions 2.0, Summit Research Services, Montrose, CO).

Results and Discussion

Three sheep from the 0.2 mg treatment died by d 19. One of these animals had ongoing difficulties with the duodenal cannula and never ate well. The other two animals exhibited symptoms including depression, emaciation, and cessation of eating. Lack of necropsy on the later two animals precluded determination of cause of death; however, given that there were no deaths on higher doses of swainsonine, it was assumed that the deaths were a result of causes other than the

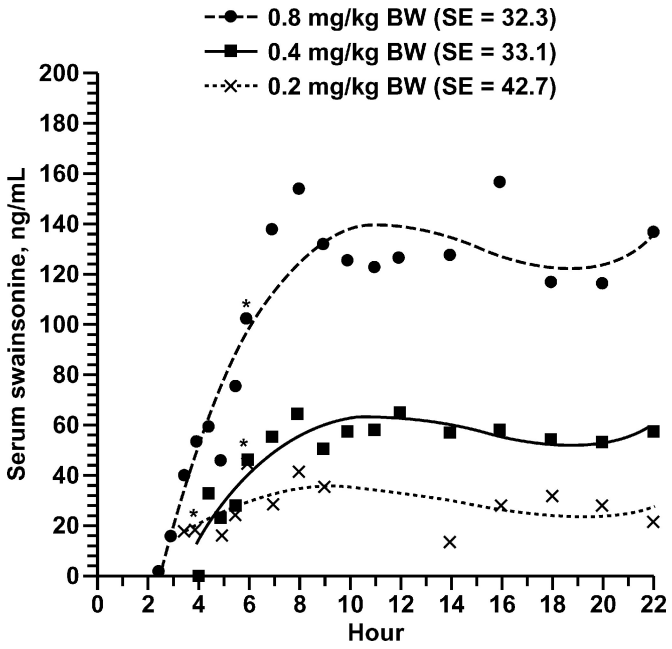

Figure 2. Dose response effects of locoweed consumption on ovine serum swainsonine activity (least squares means plotted) during the initial 22 h following initial locoweed exposure. Treatments were 0.2 (n = 2), 0.4 (n = 4), and 0.8 (n = 4) mg of swainsonine/kg of BW. *Denotes the first sampling time at which all animals within treatment had detectable concentrations of swainsonine in their serum.

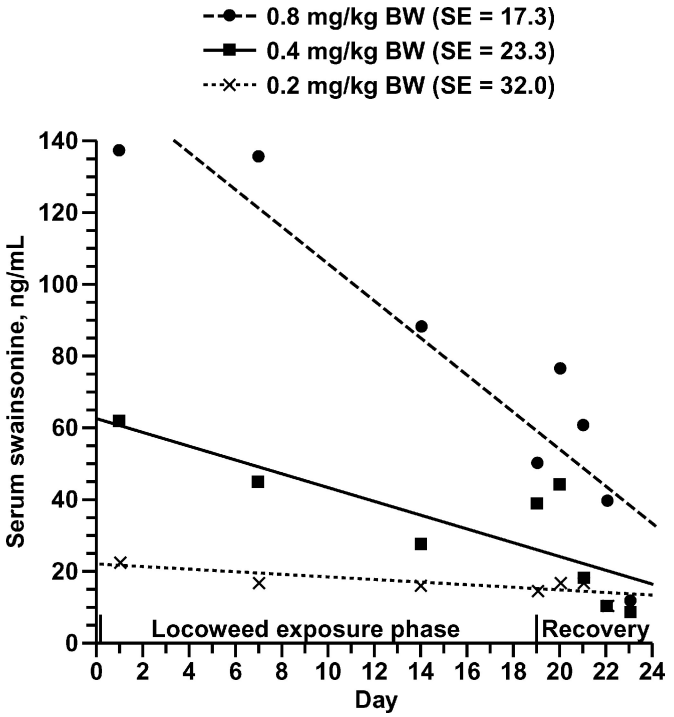

Figure 3. Dose response effect of locoweed consumption on daily ovine serum swainsonine activity (least squares means plotted) over a 19-d exposure and 5-d recovery period. Treatments were 0.2 (n = 2), 0.4 (n = 4), and 0.8 (n = 4) mg of swainsonine/kg of BW.

treatments and the animals were removed from all analyses.

Serum Swainsonine

No treatment × time interaction (P = 0.99, Figure 2) was detected for serum swainsonine for the DAY 0 intensive sampling period. Mean serum concentrations were 19.5 ± 15.8, 35.7 ± 11.1, and 103.1 ± 10.3 ng of swainsonine/mL for the 0.2, 0.4, and 0.8 mg treatments, respectively. Means separation were 0.8 vs. 0.4 (P = 0.003), 0.8 vs. 0.2 (P = 0.003), and 0.4 vs. 0.2 (P = 0.42). Linear (P = 0.03), quadratic (P = 0.008), and cubic (P = 0.02) effects across time were noted for each exposure level. Time at which serum swainsonine was first detected in all experimental animals was 3.5, 6, and 6 h for the 0.2, 0.4, and 0.8 mg swainsonine treatments, respectively. Throughout the intensive sampling period, swainsonine concentration responded in a cubic (P = 0.02) fashion, and maximal concentrations occurred by 16 h for all treatments. The lack of detectable serum swainsonine in the 0.2 mg treatment between 10 and 14 h was likely a result of assay sensitivity (6 ng/mL), and/or a depletion of available swainsonine in the gastrointestinal tract for absorption. The subsequent detectable levels following this void may indicate a return of swainsonine to the serum from deep and peripheral tissues and/or enterohepatic circulation. Following subacute locoweed exposure, Stegelmeier et al. (1995) reported serum swainsonine concentrations to be 0.395 mg/mL in sheep consuming 1.5 mg of swainsonine/kg of BW. These values are greater than the values we observed. Stegelmeier et al. (1995) collected the serum in those studies within 12 h of locoweed consumption. Taylor et al. (2000), Taylor and Strickland (2002), and Whittet et al. (2002) also reported serum swainsonine concentrations in sheep following acute locoweed exposure to be lower than those observed by Stegelmeier et al. (1995). The differences in serum swainsonine concentrations may be due to differences in the assay or the method of locoweed administration.

No treatment × time interaction was detected for serum swainsonine (P = 0.99; Figure 3) over the course of the treatment (d 0 to 19) period. Serum swainsonine was greater (P < 0.01) for the 0.8 mg treatment (80.4 ± 9.6 ng/mL) than for both the 0.4 (27.4 ± 9.9 ng/mL) and 0.2 (5.9 ± 14.2 ng/mL) treatments. Interestingly, there was a linear decrease in serum swainsonine activity as the trial progressed for the 0.8 (slope = –5.1 $ng \cdot mL^{-1} \cdot d^{-1}$; P = 0.001) and 0.4 (slope = –1.9 $ng \cdot mL^{-1} \cdot d^{-1}$; P = 0.06) mg treatments. This may indicate a change in the rate of absorption, rate of elimination, tissue distribution, serum carrying capacity, and/or an adaptive metabolism of swainsonine (e.g., liver biotransformation mechanisms or microbial population

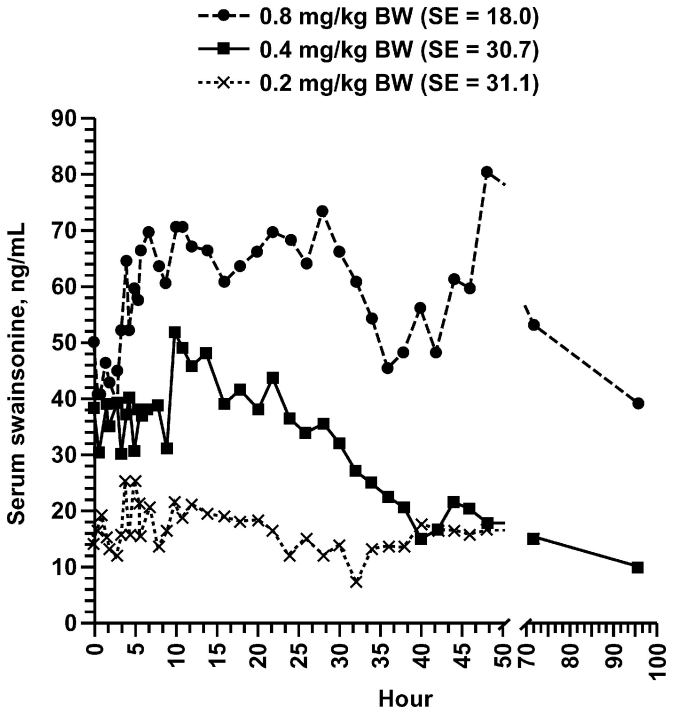

Figure 4. Clearance of swainsonine activity from ovine serum over a 96-h period following the last offering of locoweed (d 19 of experiment) in the diet (least squares means plotted). Treatments were 0.2 (n = 2), 0.4 (n = 4), and 0.8 (n = 4) mg of swainsonine/kg of BW.

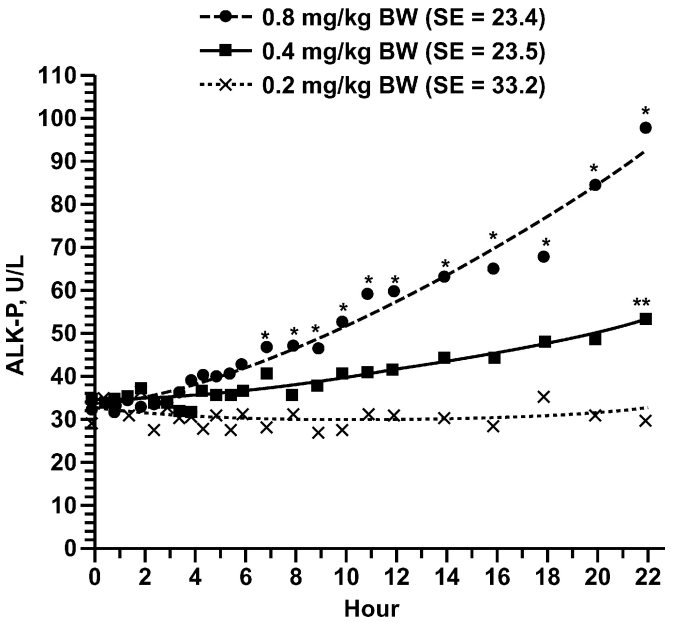

Figure 5. Dose response effects of acute exposure of swainsonine on ovine serum alkaline phosphatase (ALK-P) activity (least squares means plotted). Treatments were 0.2 (n = 2), 0.4 (n = 4), and 0.8 (n = 4) mg of swainsonine/kg of BW (0 h is baseline value; i.e., served as control). *Different ($P < 0.05$) from 0 h. **Different ($P < 0.12$) from 0 h.

shifts). If this effect is real, it has broad implications for both its effects as a toxicant and the utilization of swainsonine as a chemotherapeutic. This effect also may indicate a need to recommend withdrawal times as well as dosing protocols on the basis of prior exposure.

No treatment × time interaction ($P = 0.95$) for serum swainsonine was observed during the DAY 20 intensive (Figure 4) sampling period. The 0.4 mg treatment seemed to exhibit exponential decay kinetics of serum swainsonine; this was not readily apparent for the other two treatment levels. As such, only the 0.4 mg treatment was used to estimate elimination and distribution rates, which were 0.012 and 0.066 h^{-1}, with half-lives of 56.7 and 10.5 h, respectively. Mean serum swainsonine concentrations were different (0.8 > 0.4 > 0.2 mg; $P = 0.09$ for protection level) among treatments and were 17.3 ± 2.8, 34.1, and 58.6 ± 1.9 ng/mL for the 0.2, 0.4, and 0.8 mg treatments, respectively. Results of this study indicate that the distribution rates of the 0.4 mg treatment were comparable to those observed by Stegelmeier et al. (1998; 10.5 vs. 15 to 20 h) for serum clearance. In addition, the elimination rate agrees with what Stegelmeier et al. (1998) estimated for the liver tissue clearance (56.7 vs. 60 h). Our data, along with those of Stegelmeier et al. (1998), support a multiple-compartment model of swainsonine disposition. The 0.8 mg treatment had sustained levels of swainsonine in the serum at 42 h; possible causes include enterohepatic circulation or return from tissues due to lysosomal trapping of swainsonine (Figure 4) after last exposure (this was less pronounced in the lower treatments). As such, there were inadequate data collected after this point to predict elimination rates from the body.

Serum Alkaline Phosphatase

A treatment × time interaction ($P = 0.02$) was detected for serum ALK-P during the DAY 0 intensive sampling (Figure 5). Serum ALK-P activity was elevated ($P < 0.001$) for the 0.8 mg treatment by 7 h, and continued to increase in a linear (rate = 3.0 + 0.56 $U \cdot L^{-1} \cdot h^{-1}$; $P < 0.001$) fashion throughout the intensive sampling. These differences were not observed for the 0.2 and 0.4 mg treatments; however, the 0.4 mg treatment followed a pattern similar to that of the 0.8 mg treatment, with the 22-h sample tending to be elevated ($P = 0.12$). Slope values for the 0.4 (different from 0, $P = 0.15$) and 0.2 mg (different from 0, $P = 0.88$) treatments were 0.82 ± 0.57 and –0.12 ± 0.80 $U \cdot L^{-1} \cdot h^{-1}$, respectively.

Over the course of the study, no treatment ($P = 0.28$) or treatment × time interaction ($P = 0.92$) was noted for ALK-P activity (Figure 6). Serum ALK-P activity was increased ($P < 0.05$) for all treatments on d 7, 14, and 21 over pretreatment and recovery period values. Quadratic ($P = 0.02$) and cubic ($P = 0.006$) effects were noted with time of locoweed exposure. Further, 24 h (d 20) into the recovery period, ALK-P activities returned toward pretreatment ($P = 0.13$) levels, with full recovery by d 26. Although serum swainsonine activities were

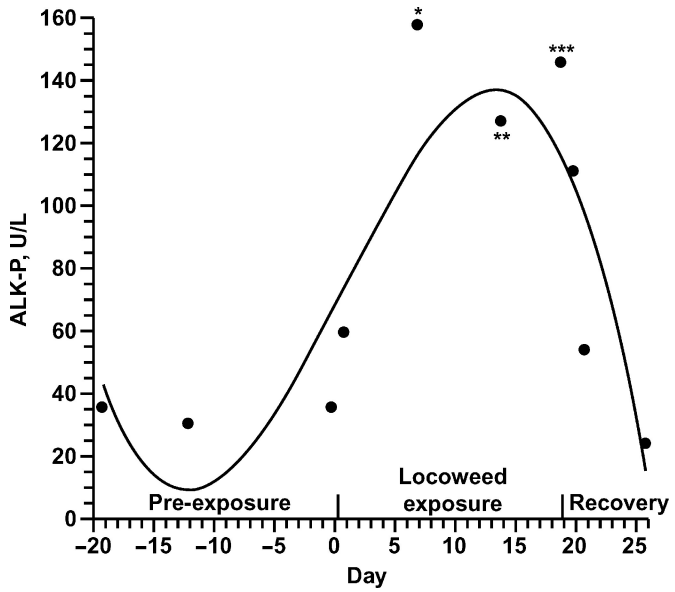

Figure 6. Effect of subacute exposure to swainsonine on ovine serum alkaline phosphatase (ALK-P) activity (least squares means plotted; n = 10; SE = 41.2). *Different ($P < 0.05$) from d –19, –12, 0, 1, 21, and 26. **Different ($P < 0.10$) from d –19, –12, 0, and 26. ***Different ($P < 0.05$) from d –19, –12, 0, 21, 26, and 1 ($P < 0.10$).

still detectable at 24 h after removal of exposure (Figures 3 and 4), the activities were at or below 80 ng/mL. This level did not correspond to elevated ALK-P during the acute exposure phase of this study (Figure 5), indicating that recovery was occurring with decreasing concentrations of swainsonine. Furthermore, these data indicate rapid recovery of the animal from the mechanism responsible for ALK-P elevation. Serum ALK-P has been shown to increase in cattle and sheep consuming locoweed (Pulsipher et al., 1994; Taylor et al., 2000; Taylor and Strickland, 2002). Additionally, serum ALK-P has been shown to increase as early as 24 h (Taylor and Strickland, 2002). Our data agree with previous studies, indicating that serum ALK-P remains elevated approximately 7 d after the cessation of subacute locoweed exposure, and returns to normal levels by 14 d (Bachman et al., 1992; Pulsipher et al., 1994; Taylor et al., 2000). The source of increased alkaline phosphatase activity has not yet been determined. Bachman et al. (1992) reported no changes in Ca, P, or indirect, direct, and total bilirubin, and speculated that no damage was done to bone, and that cholestasis did not occur. They suggested that the source of increased ALK-P is hepatocytes (Bachman et al., 1992). Vacuolization of hepatocytes reported by VanKampen and James (1969) would also support the suggestion that the source of increased ALK-P is hepatocytes; however, an increase in total and indirect bilirubin concentrations should have been observed if hepatocyte death had occurred. We have speculated that the increased serum ALK-P activity during the acute exposure period is due to altered glycoprotein processing (e.g., gut, kidney, and liver) induced by swainsonine rather than due to cell death caused by lysosomal vacuolation of the tissues. Evidence of this stems from the lack of consistent concomitant changes in corresponding cell death markers observed here (see Figure 8) and in other studies (Taylor et al., 2000; Whittet et al., 2002) immediately following acute exposure. As noted previously, however, the exact tissue origin of serum ALK-P activity remains to be determined.

Another explanation for the increases in serum ALK-P activity could be the stress placed on the animals due to surgery and manipulation (Kramer and Hoffmann, 1997). However, two lines of evidence argue against this being the mode of the observed elevated serum ALK-P activities in sheep exposed to swainsonine via locoweed. The first is the data presented in Figure 6 showing that on d –19, –12, and 0 (i.e., baseline control), sheep did not have elevated levels of serum ALK-P. This period followed a 30-d recovery period after surgeries to place ruminal and duodenal cannulas were performed. As such, the sheep were allowed approximately 51 d to recover (30 d surgical recovery followed by a 21-d dietary and crate adjustment period) before swainsonine exposure via locoweed on d 1 of the locoweed exposure period. If the surgical, adaptation, and handling stresses were the cause of the elevated ALK-P activities, then the activities should have been elevated before swainsonine exposure.

The second line of evidence is provided by inclusion of Figure 7. Data presented in Figure 7 was taken from a swainsonine toxicokinetic experiment, in which 15 wethers (74.2 ± 4.2 kg) were housed outside in individual pens (4 m × 1.5 m), stratified by BW, and assigned to one of three treatments. Treatments were: 0 (n = 5), 0.4 (n = 5), and 1.6 (n = 5) mg of swainsonine/kg of BW delivered via locoweed extract. Animals were allowed a 14-d adaptation to a blue grama hay basal diet (1.7% BW on DM basis), with the diet formulated to meet maintenance requirements (TDN and CP; NRC, 1985) before swainsonine exposure. Immediately before the start of intensive sampling, swainsonine was delivered orally via syringe as a crude locoweed (*Oxytropis sericea*) extract mixed with corn syrup (15 to 20 mL). A jugular serum sample was collected just before dosing, followed by sampling after dosing at 1-h intervals from 0 to 12 h, 3-h intervals from 15 to 24 h, 6-h intervals from 30 to 48 h, and 12-h intervals from 60 to 168 h. Animals in this experiment were not surgically altered in any manner. As shown in Figure 7, serum ALK-P activity increased following swainsonine exposure for the 1.6 mg treatment (6 through 96 h) and to some extent the 0.4 mg treatment (12 and 30 h). Note that similarly treated and handled control wethers (0 mg treatment) did not exhibit elevated serum ALK-P activities. This clearly demonstrates that swainsonine, and not handling stress or surgical procedures, was responsible for the elevated ALK-P activities. However, these data do not rule out the possibility that swainsonine exposure caused a direct increase (i.e., other than

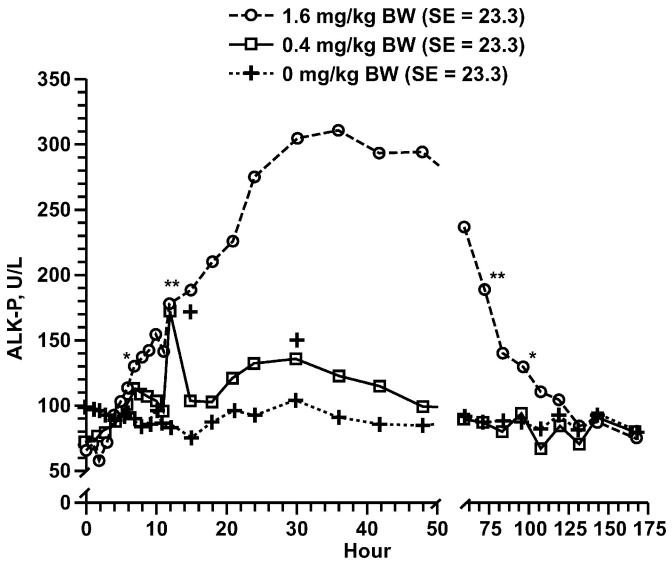

Figure 7. Dose response effects of acute oral exposure of swainsonine (delivered via locoweed extract) on serum alkaline phosphatase (ALK-P) activity in sheep not surgically altered. This graph is of data from another experiment (not reported herein) as an illustration that surgical and handling stresses were not the cause of elevated serum ALK-P activity in the present study. Treatments were 1.6, 0.4, and 0 mg of swainsonine/kg of BW. Time 0 h was collection immediately before extract administration. *Times 6 through 96 h for the 1.6 mg treatment were different ($P < 0.05$) than 0 h. +Times 12 and 30 h for the 0.4 mg treatment were different ($P < 0.05$) than 0 h. **Times 12 through 72 h for the 1.6 mg treatment were different ($P < 0.05$) than corresponding times for the 0.4 and 0 mg treatments.

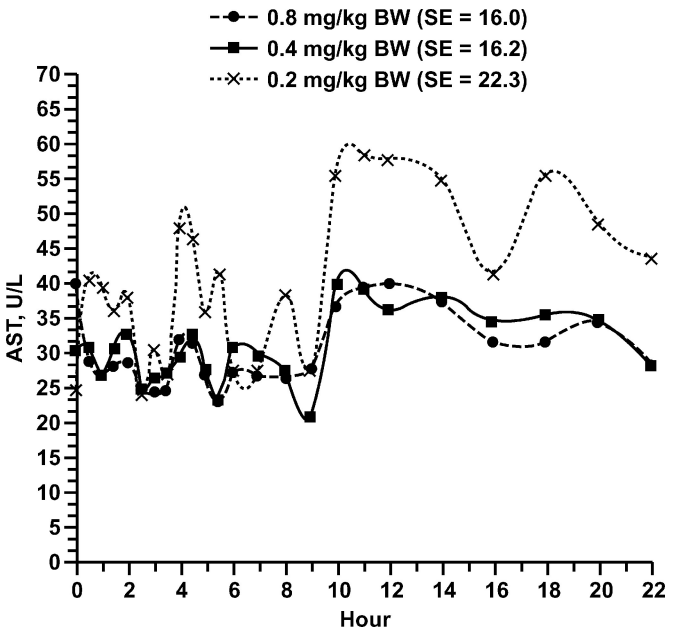

Figure 8. Dose response effects of acute exposure of swainsonine on ovine serum aspartate aminotransferase (AST) activity (least squares means plotted). Treatments were 0.2 (n = 2), 0.4 (n = 4), and 0.8 (n = 4) mg of swainsonine/kg of BW (0 h is the baseline value; i.e., served as control).

stresses already discussed) in cortisol concentrations that resulted in the increased ALK-P activities.

Serum Aspartate Aminotransferase

Serum AST activities in our study exhibited a treatment × time interaction ($P = 0.04$; Figure 8) during the DAY 0 intensive sampling period. However, upon examination of the means within time, no differences among treatments were detected ($P > 0.30$). Furthermore, for the most part, serum AST levels did not deviate from 0 h ($P > 0.10$) over the intensive sampling period within treatment. In support of this statement, no linear ($P = 0.98$), quadratic ($P = 0.63$), or cubic effects ($P = 0.11$) of swainsonine with time from exposure were noted for any treatment; however, several time points in the 0.2 mg treatment approached significance ($P < 0.10$) from 0 h. These included 0.5, 1, 4, 4.5, 10, 11, and 12 h. It is possible, given that the values varied considerably over the sampling period, that red blood cell lysis in some samples may have contribute to the elevated levels. The apparent lack of change in serum AST in the 0.4 and 0.8 mg treatments support the previous speculation that cellular necrosis was not occurring during the acute exposure period.

There was no swainsonine level ($P = 0.67$) or swainsonine level (day of exposure effect ($P = 0.42$); however, linear ($P = 0.005$) and cubic ($P = 0.06$) effects were noted with time of locoweed exposure. Similar to serum ALK-P activities, serum AST activities were also increased ($P < 0.05$) across treatment levels on d 7, 14, 19, 20, 21, and 26 over baseline (Figure 9). However, serum AST activities did not return to pretreatment baseline values, indicating that tissue damage (i.e., cell leakage) could still have been occurring. Pulsipher et al. (1994), Taylor et al. (2000), and Whittet et al. (2002) reported similar results. Due to potential trapping of swainsonine within the lysosome (Dorling et al., 1980), swainsonine-induced lysosomal storage disease may continue to occur for a period after withdrawal from locoweed. Because swainsonine does not cause vacuolization of skeletal or cardiac muscle (James et al., 1970), increases in serum AST may be the result of vacuolization of neural, hepatic, or renal tissue.

Serum Iron and Unsaturated Iron Binding Capacity

No treatment ($P = 0.12$) or treatment × time effects ($P = 0.99$) were detected for serum Fe during the DAY 0 intensive sampling period (Figure 10), over the course of the study (Figure 11), or during the DAY 20 intensive sampling period. However, when treatment data were combined within day and the model analyzed only for

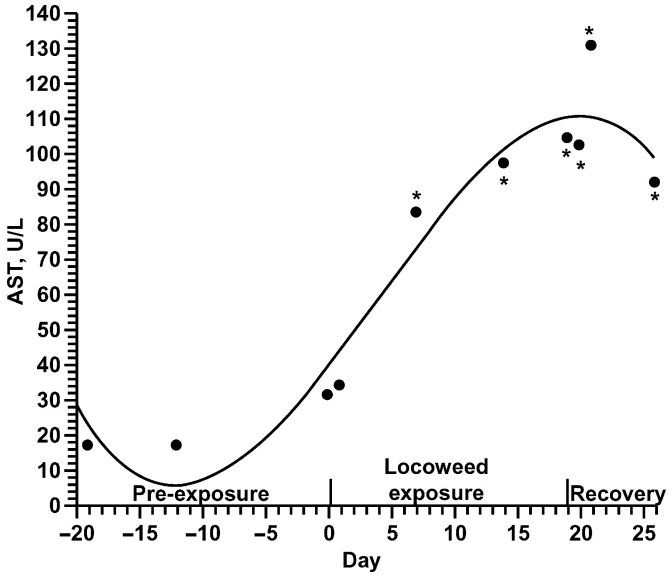

Figure 9. Effect of subacute exposure to swainsonine on ovine serum aspartate aminotransferase (AST) activity (least squares means plotted; n = 10; SE = 17.9). *Different ($P < 0.05$) from d –19, –12, and 0 (i.e., baseline values).

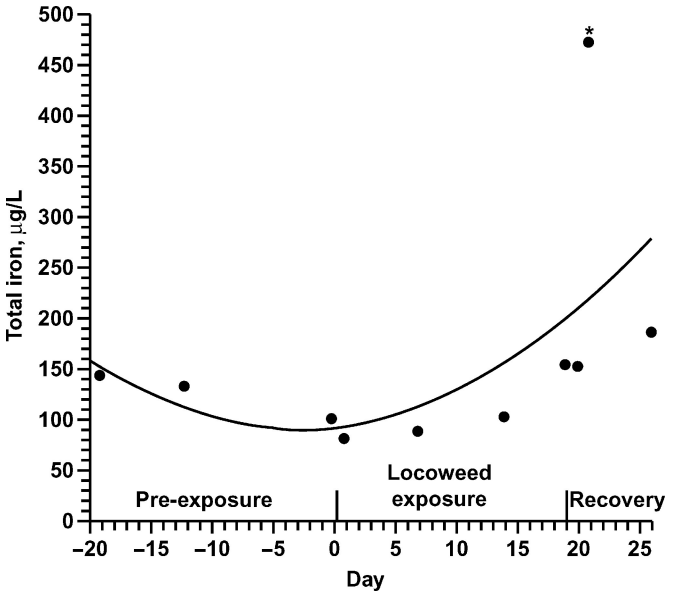

Figure 11. Effect of subacute exposure to swainsonine on ovine serum total iron (μg/L) concentration (least squares means plotted; n = 10; SE = 56.5). *Different ($P < 0.05$) from all other sampling dates.

a time effect during the DAY 0 intensive sampling period, a linear decrease (slope = –1.23 $mg \cdot L^{-1} \cdot h^{-1}$; $P = 0.001$) in serum Fe was detected. More convincing evidence that Fe metabolism may have been altered by swainsonine exposure is demonstrated by the quadratic ($P = 0.018$) increase in serum Fe during the recovery period (d 20 to 30; Figure 11). We speculate that the suppressive effects of locoweed on serum Fe are due to the inhibition of Fe mobilization from the liver or effects on intestinal transport. In support, accelerated cytosolic transport and secretion of transferrin has been reported when hepatoma cells are treated with swainsonine in vitro (Yeo et al., 1985). Furthermore, Galyean et al. (1996) reported higher liver Fe concentrations in animals consuming locoweed. This evidence further suggests that the liver is sequestering Fe, and there is a problem with mobilizing that Fe from the liver, leading to altered Fe transport as a reason for decreased serum Fe concentrations. Furthermore, Fe balance is largely regulated at the level of the small intestine through control of absorption rates (Wood and Han, 1998). Several of the proteins involved in Fe transport across enterocytes are reported to be glycoproteins offering the potential for swainsonine to alter Fe absorption through alterations in glycoprotein structure. These alterations could then potentially decrease Fe concentrations through decreased intestinal absorption of Fe. Other studies have reported similar and more dramatic effects on serum Fe, both acutely (Taylor et al., 2000) and subacutely (Bachman et al., 1992). These rapid changes in Fe metabolism indicate a need to worry about the potential acute effects of swainsonine on the efficiency of metabolism of other nutrients in the short term.

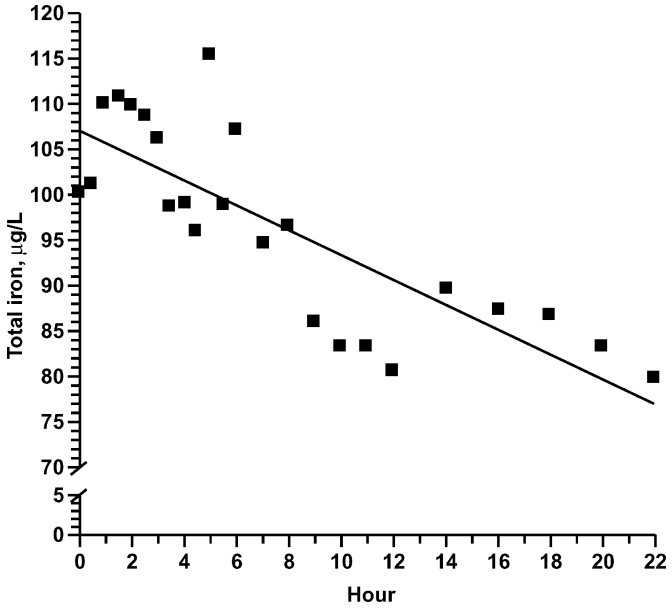

Figure 10. Linear (slope = –1.23 $\mu g \cdot L^{-1} \cdot h^{1}$); $P = 0.001$) decrease in ovine serum total iron concentration (least squares means plotted; n = 10; SE = 9.6) following acute swainsonine exposure, with 0 h serving as the baseline control value.

There was no treatment level ($P = 0.15$), time of exposure ($P = 0.39$), or treatment level × time of exposure effect ($P = 1.0$) for serum UIBC during the DAY 0 intensive sampling period (Figure 12), over the course of the study (Figure 13), or during the DAY 20 intensive sampling period. Likewise, no deviations from the pre-

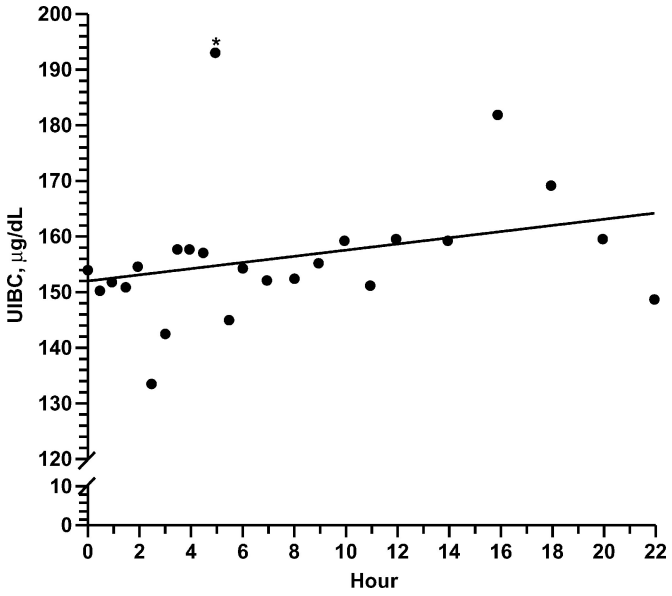

Figure 12. Effect of acute exposure to swainsonine on ovine serum unsaturated iron binding capacity (UIBC; least squares means plotted; n = 10; SE = 13.5) with 0 h as the control value. *Different from 0 h.

treatment baseline occurred within treatment over the course of the study. We originally hypothesized that UIBC might change as evidenced by earlier data indicating that Fe metabolism is altered in animals consuming locoweed (Bachman et al., 1992; Galyean et al., 1996; Taylor et al., 2000). As discussed previously, accelerated cytosolic transport and secretion of transferrin has been reported when hepatocytes are treated with swainsonine in vitro (Yeo et al., 1985), leading us to speculate that altered Fe transport is the reason for decreased serum Fe; however, we found no change in UIBC for any treatment in the study.

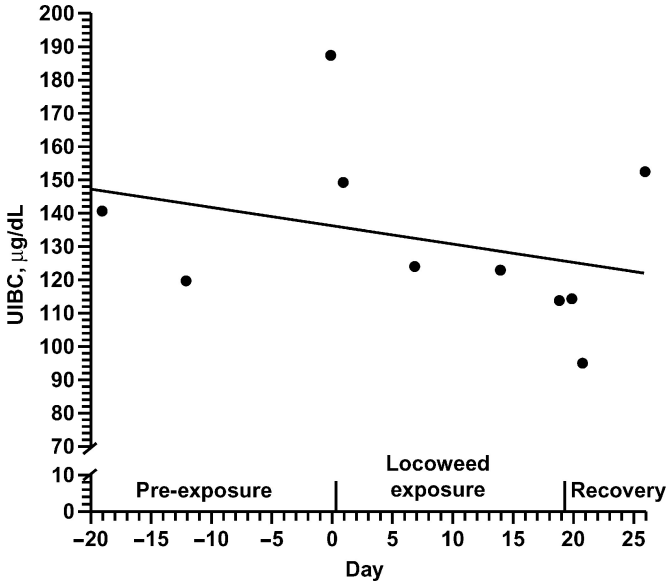

Figure 13. Effect of subacute exposure to swainsonine on ovine serum unsaturated iron binding capacity (UIBC; least squares means plotted; n = 10; SE = 23.7).

Serum Ceruloplasmin, Triglycerides, and Cholesterol

Serum ceruloplasmin did not change with increasing swainsonine ($P = 0.49$), nor was there a treatment × time ($P = 0.99$) effect noted for ceruloplasmin over the course of the study (Figure 14). A time effect was noted, whereby serum ceruloplasmin levels were lower on d 14 ($P = 0.05$) and 19 ($P = 0.07$; Figure 13) than pretreatment baseline values; this effect was cubic ($P = 0.001$), supporting a potential suppression of ceruloplasmin activity by swainsonine. Ceruloplasmin has not been extensively studied with regard to locoweed toxicity. As with transferrin, accelerated cytosolic transport and secretion of ceruloplasmin has been reported when hepatocytes are treated with swainsonine in vitro (Yeo et al., 1985). This might be expected due to the fact that ceruloplasmin is a glycoprotein and swainsonine alters glycoprotein processing (Yeo et al., 1985; Broquist, 1986). Interestingly, Galyean et al. (1996) reported that liver Cu concentrations, unlike Fe, were lower in animals consuming locoweed. Recovery of ceruloplasmin levels in our study coincide with similar changes in serum Fe. Our findings coupled with those of Galyean et al (1996) and Yeo et al. (1985) indicate that subclinical effects of swainsonine intoxication may affect nutrient metabolism and animal production without clinical expression of locoism.

There was no treatment ($P = 0.74$) or treatment × time ($P = 0.42$) effect noted for serum triglyceride levels during this experiment (Figure 14); however, there was a linear increase (slope = 0.33 $mg \cdot dL^{-1} \cdot d^{-1}$; $P = 0.01$) in triglycerides over time after the initial d 0 swainsonine exposure. Serum triglyceride concentrations have been shown to increase for control animals, while decreasing or remaining constant by d 14 and 28 of locoweed ingestion in cattle (Bachman et al., 1992). The reason for changes in triglyceride concentration is unknown; however, we speculate that serum triglyceride may be altered through glucosidase inhibition of low-density lipoprotein receptor expression on smooth muscle cells (Elbein, 1989). Even though low-density lipoprotein receptor expression may be only slightly affected by swainsonine (Elbein, 1989), total hepatic triglyceride output might be affected by swainsonine α-mannosidase enzymes and thereby alter the activity of enzymes involved in triglyceride, lipoprotein, or lipoprotein receptor synthesis. It is possible that the dietary makeup (i.e., locoweed vs. alfalfa in the diet) might have influenced triglyceride levels in the present study.

There were no effects of treatment ($P = 0.44$), time ($P = 0.09$), or treatment × time ($P = 0.52$) noted for serum cholesterols concentrations (Figure 14) during the course of the study. Furthermore, there were no linear ($P = 0.46$), quadratic ($P = 0.80$), or cubic ($P = 0.51$), effects from time of exposure noted for cholesterol

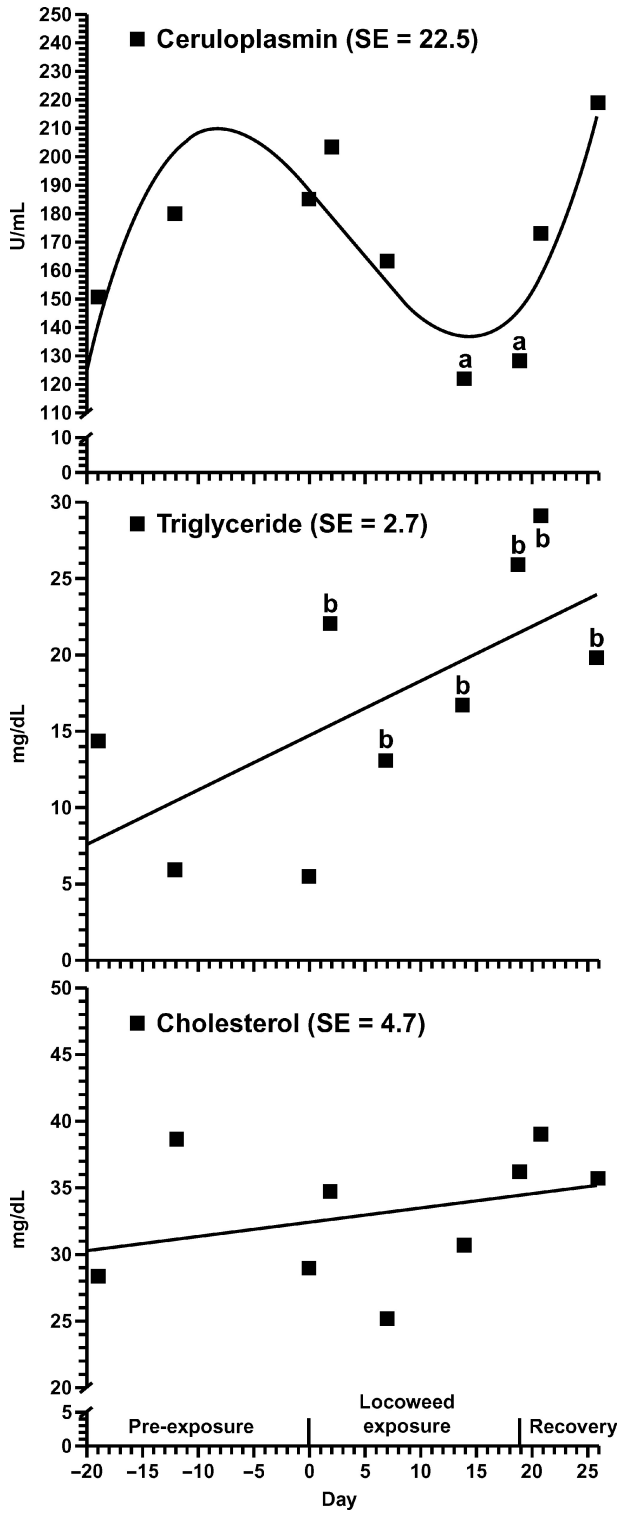

Figure 14. Effects of subacute exposure to swainsonine on ovine serum ceruloplasmin, triglyceride, and cholesterol concentrations (least squares means plotted; n = 10). Ceruloplasmin: [a]different ($P < 0.07$) from d –12 and 0 (baseline values; i.e., control values); cubic ($P = 0.001$) effect with day of exposure. Triglyceride: [b]different ($P < 0.06$) from d –12 and 0 (baseline values; i.e., control values); linear (slope = 0.33 $mg \cdot dL^{-1} \cdot d^{-1}$); $P = 0.001$) effect with day of exposure. Cholesterol: no effects of swainsonine were noted.

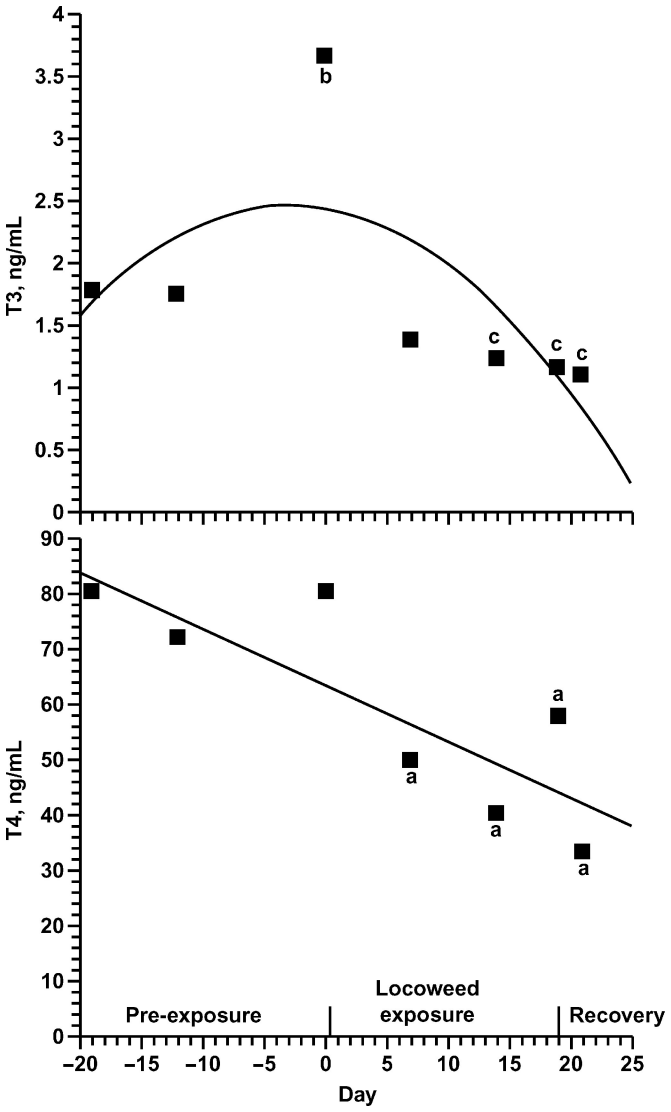

Figure 15. Effects of subacute exposure to swainsonine on ovine serum triiodothyronine (T3) and thyroxine (T4) concentrations (least squares means plotted; n = 10; T3 – SE = 0.24; T4 – SE = 5.8). T3: [b]different ($P = 0.001$) from d 0 (baseline value; i.e., control value); [c]different ($P < 0.13$) than d –19, and –12 (baseline values; i.e., control values); linear (slope = –0.02 $ng \cdot mL^{-1} \cdot d^{-1}$; $P < 0.06$) and quadratic ($P = 0.001$) effects of day of exposure. T4: [a]different ($P < 0.10$) from d –19, –12, and 0 (baseline values, i.e., control values); linear ($P = 0.001$).

(Figure 14). These findings agree with previous data, where consumption of less than 1.6 mg of swainsonine/kg of BW did not seem to alter serum cholesterol (Taylor et al., 2000).

Serum Triiodothyronine and Thyroxine

There were no treatment ($P = 0.80$) or treatment × time ($P = 0.99$) effects for serum T3 (Figure 15). Linear ($P = 0.05$) and quadratic ($P = 0.0009$) effects of time of exposure were noted for serum T3 concentrations. No

treatment × time of exposure ($P = 0.43$) interaction for serum T4 concentrations was noted; however, there was an apparent dose response effect ($P = 0.04$ for protection level; 70.1 ± 7.1, 45.4 ± 5.0, 63.5 ± 5.0 ng/mL for 0.2, 0.4, and 0.8 mg treatments, respectively) of swainsonine. Further, a negative linear ($P = 0.0001$) effect of time was noted for serum T4 throughout the course of the study. Previous studies reported that locoweed consumption could alter the production of T3 and T4 hormones (Pulsipher et al., 1994; Richards et al., 1999). These studies found that T3 and T4 concentrations decreased 7 d after the consumption of locoweed. In the present study, the concentrations of T3 and T4 followed the same trend, with decreasing serum T3 and T4 concentrations after consumption of locoweed. This finding would not be unexpected, given that the precursor to and storage form of the thyroid hormone, thyroglobulin, is in fact a glycoprotein (Kaneko, 1997). Thus, synthesis of thyroglobulin could be affected by the action of swainsonine on the Golgi pathway. However, thyroglobulin is converted to T3 and T4 utilizing lysosomal proteases of the thyroid follicular cells, thereby providing another opportunity through inhibition of lysosomal mannosidase to impair T3 and T4 production. The poor health and production of animals consuming locoweed is likely to be partially related to the effect of swainsonine on thyroid hormones. Given that initial serum T4 levels 25 to 30 ng/mL were lower in the 0.4 mg treatment than the 0.8 and 0.2 mg treatments, and there was no interaction of dose with day of exposure, the apparent dose response effects seen here likely reflect the early difference that is unrelated to the swainsonine treatment.

In conclusion, because certain serum constituents, such as alkaline phosphatase, change within 22 h following first exposure, and considering that cell death and tissue damage do not occur until several days after locoweed ingestion, we speculate that acute changes are the result of altered glycoprotein processing and not cytotoxicity. Therefore, it is plausible that a "no-effect level" of swainsonine consumption would allow producers to develop a safe management protocol for locoweed-infested areas. Based on our observations and those of Taylor et al. (2000), if a no-effect level exists, it will be below 0.2 mg of swainsonine/kg of BW.

Implications

Sheep ingesting 0.2 mg of swainsonine/kg of body weight showed minimal subclinical changes. As such, it may be possible to allow animals to graze locoweed-infested areas for short periods, as long as consumption does not regularly exceed 0.2 mg of swainsonine/kg of body weight daily. Nonetheless, until a full accounting of the acute effects of the toxicant is made, care should be exercised in making recommendations concerning limit grazing.

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